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Effects of Exercise Training on the Blood Lactate Response to Acute Exercise in Cancer Survivors

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EFFECTS OF EXERCISE TRAINING ON THE BLOOD LACTATE RESPONSE TO ACUTE EXERCISE IN CANCER SURVIVORS

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ABSTRACT

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Otto Warburg, a German physiologist, deduced that cancer cells exhibit a remarkably different metabolic profile in comparison to normal healthy cells characterized by increased rates of glycolysis and lactate fermentation, even in the presence of adequate oxygen content. Through the respective gain of function/loss of function of oncogenes and tumor suppressor genes, three major genes emerge as hallmarks of carcinogenic metabolic reconfiguration; HIF-1a, c-Myc oncogenes, and p53 tumor-suppressor genes. A result of their respective gain/loss of function are the upregulation of several glycolytic proteins and subsequent high rates of glucose influx into the tumor microenvironment, as well as lactate production, accumulation, and extrusion out of the tumor microenvironment. Patterns of lactate accumulation in apparently healthy models upon a progressive exercise bout before and after an exercise intervention exist in the structure of Resting, Lactate Thresholds 1 & 2 (LT1/LT2), Onset of Blood Lactate Accumulation (OBLA), and Peak parameters. However, it is unclear how the structure of these parameters applies to a cancer population and further, between cancer survivors actively receiving cancer therapy and cancer survivors who are not. **Purpose:** To investigate blood lactate accumulation response to a progressive exercise bout to volitional fatigue before and after a 12-week exercise-based oncology rehabilitation intervention between active and inactive cancer survivors. **Methods:** 21

cancer survivors (active, n=7; inactive, n=14) were invited to participate in a 12 week-week exercise-based oncology rehabilitation program at the University of Northern Colorado Cancer Rehabilitation Institute. All participants performed an initial assessment consisting of several physiological parameters, including body composition, pulmonary function, balance, muscular strength, muscular endurance, cardiovascular fitness, and lactate accumulation (LA). LA was quantified every 2 minutes during a progressive treadmill protocol to volitional fatigue via finger stick. The 12-week exercise intervention consisted of one-hour exercise sessions with trained Cancer Exercise Specialists focusing on cardiovascular endurance, muscular strength and endurance, balance, and flexibility exercises. Following the 12 week-exercise based intervention, participants completed a reassessment of the same physiological parameters. **Results:** Resting, Peak, and METS at OBLA values of active and inactive cancer survivors before and after an exercise intervention were not different ($P>0.05$). Furthermore, Resting lactate, Peak lactate, and METS at OBLA values between active and inactive cancer survivors before and after an exercise intervention were insignificantly different ($P>0.05$). However, both METS at OBLA in both active and inactive survivors were significantly different before and after a 12-week exercise intervention ($P<0.05$). **Conclusions:** These findings may indicate similar responses of lactate handling to exercise training in both active and inactive cancer survivors. This outcome is supported by significance of increased METS at OBLA of the total subject population before and after a 12-week exercise intervention indicating an improved ability to buffer lactate at higher intensities during the UNCCRI treadmill protocol.

TABLE OF CONTENTS

LIST OF TABLES

Table

LIST OF FIGURES

Figure

CHAPTER I

INTRODUCTION

Cancer, according to the American Cancer Society, is a disease defined by abnormal, uncontrolled cell division and its respective spread to other parts of the body through the blood and lymph systems. Several million people are diagnosed with cancer each year and that number is expected to continue to increase over the next decade (Bray et al., 2017). It is evident that cells naturally consume energy in order to sustain rates of proliferation and normal healthy cells have highly orchestrated pathways to efficiently recycle metabolites produced as a result of energy utilization. Lactate, ultimately as a production of glucose metabolism, is typically shuttled to cells highly capable of oxidative phosphorylation, where it is reduced to pyruvate and utilized as an intermediate through the Krebs Cycle (Mougios, 2006). In addition, lactate itself is a source of energy as well as a signaling molecule for various metabolic adaptations. Cancer cells behave differently in that they metabolically reconfigure themselves to support the dysregulation of lactate metabolism in an effort to provide a conducive environment pertinent to cancer development and progression.

Otto Warburg, a German physiologist, noted several decades ago that cancer cells exhibit a remarkably different metabolic profile in comparison to normal healthy cells characterized by increased rates of glycolysis and lactate fermentation, even in the presence of adequate oxygen content (Warburg, Wind, & Negelein, 1927). Lactate, as a key player in cancer, was dismissed for several decades after Warburg's proposed

hypothesis until it was recently reintroduced as a metabolite involved with cancer signaling mechanisms. Apparently healthy cells must exhibit some aspect of genetic mutation in order to be classified as a cancerous cell. These mutations can be a result of hereditary and/or environmental influences, however it is necessary for a cell to experience two separate "hits" to be classified as a cancerous cell, as according to the "multiple hit theory" (Ashley, 1969). Cells contain oncogenes, which aid in the regulation of genes and proteins necessary for cell growth, and tumor-suppressor genes, which aid in halting cell proliferation and promoting apoptosis or "programmed cell death" (Fearon & Bommer, 2008). Together, these genes aid in the regulation and prevention of these carcinogenic hits and it is when these genes experience a respective gain (GOF) or loss of function (LOF) due to hereditary or environmental influences, they become dysregulated, thus promoting abnormal and uncontrolled cell division (Ringer & Schnipper, 2001). HIF-1α and p-53 oncogenic GOF along with c-Myc LOF are believed to play a role in carcinogenic regulatory mechanisms and have consistently been associated with cancer aggressiveness and poor prognosis (Brizel et al., 2001; Kunkel et al., 2003; Unruh et al., 2003; Younes, Brown, Stephenson, Gondo, & Cagle, 1997)

Together, the respective GOF and LOF of these oncogenes and tumor-suppressor gene have led to the identification of important steps related to lactagenesis, the production of lactate, and its subsequent relation to cancer aggressiveness. The mutation of these regulatory genes first leads to an increased expression and activity of glycolytic enzymes including glucose transporters (GLUT), catabolic glycolytic intermediates such as pyruvate dehydrogenase kinases and lactate dehydrogenase enzymes, and its successive decrease in mitochondrial function, thereby preventing oxidation of

metabolites for energy synthesis through the Krebs cycle (De Saedeleer et al., 2012; Firth, Ebert, & Ratcliffe, 1995; Levine & Puzio-Kuter, 2010; Osthus et al., 2000). Lactate produced as a result of anaerobic glycolysis, even in the presence of oxygen, is not shuttled into the mitochondria for oxidative phosphorylation through the Krebs cycle but rather accumulates and is extruded via the upregulation of monocarboxylate transport proteins (Shi et al., 2001). This extrusion of lactate and its associated hydrogen proton reduces pH levels of the tumor microenvironment and stimulates acidosis-induced upregulation of vascular endothelial growth factor (VEGF), leading to increased rates of glucose delivered to the tumor itself (Polet & Feron, 2013; Sonveaux et al., 2012). Brizel et al., (2001) noted that blood lactate concentrations of cancer survivors were up to 40% higher in 34 newly diagnosed cancer patients than that of an apparently healthy population and that these high blood lactate concentrations were associated with the subsequent development of distant metastasis and tumor aggressiveness. This cycle of increased glycolytic enzyme expression/activity, glucose uptake and subsequent lactate production, accumulation, and release is constantly repeated throughout the process of tumorigenesis, which explains the findings of high lactate levels of cancer cells and its association with cancer metastasis and poor prognosis.

Recent studies investigated the effects of exercise on gene structure and integrity to modulate the mutations of proto-oncogenes and tumor-suppressor genes associated with carcinogenesis and related lactate production (Abe et al., 2015; Gohil & Brooks, 2012; Safdar et al., 2015). Exercise facilitates proper production, accumulation, and disposal of lactate and the upregulation of lactagenic intermediates in apparently healthy populations, however little is known regarding exercise-mediated effects of cancer

metabolism. As exercise has been used to modulate cell-to-cell transport mechanisms of glucose and lactate in diabetic models (Juel, Holten, & Dela, 2004), it is plausible to infer that exercise may regulate similar mechanisms in cancer survivors.

Statement of Purpose

The purpose of this study was to compare blood lactate accumulation response to a progressive exercise bout to volitional fatigue before and after a 12-week exercise-based oncology rehabilitation intervention between cancer survivors currently undergoing cancer treatment and cancer survivors not undergoing cancer treatment.

Research Hypotheses

H1 Cancer survivors in treatment will have lower resting blood lactate levels than cancer survivors out of treatment before and after a 12-week exercise intervention.

H2 Cancer survivors in treatment will reach OBLA at a lower METS than cancer survivors out of treatment before and after a 12-week exercise intervention.

H3 Cancer survivors in treatment will have lower peak blood lactate accumulation levels than cancer survivors out of treatment before and after a 12-week exercise intervention.

Need for the Study

As cancer rates and research on cancer as a metabolic disease continue to rise, comprehending the aspect of cancer metabolism becomes all the more prevalent. It is clear that oncogene and tumor-suppressor gene dysregulation lead to increased rates at which tumors metabolize carbohydrates and subsequent dysregulated lactate handling (Boidot et al., 2012; Sonveaux et al., 2012; Ullah, Davies, & Halestrap, 2006). Ultimately, dysregulated lactate handling in cancer survivors with HIF-1α/c-Myc GOF and/or p53 LOF results in lactate mediated angiogenesis via VEGF (Polet & Feron, 2013;

Sonveaux et al., 2012). Research in cancer biology lacks information on blood lactate parameter response to an exercise intervention. Since lactate production, accumulation, and disposal may be regulators of cancer aggressiveness and key indicators of poor prognosis, it is pertinent to understand how lactate accumulates in cancer survivors. There are currently no studies that have investigated blood lactate parameters during a progressive exercise bout to volitional fatigue in cancer survivors and it is unclear how those parameters are affected by a 12-week exercise-based oncology rehabilitation intervention.

CHAPTER II

REVIEW OF LITERATURE

Cancer, one of the leading causes of death worldwide, is characterized as "a disease in which abnormal cells divide without control and… spread to other parts of the body through the blood and lymph systems" (National Program of Cancer Registries, 2018). Over 18 million people were diagnosed with cancer in 2018 according to incidence data compiled by the World Health Organization and International Agency for Research on Cancer, (Forman, Ferlay, Stewart, & Wild, 2014). As the mechanisms of cancer start to become more heavily explored, one clear characteristic that continues to emerge is the aspect of cancer metabolism and its anabolic/catabolic function(s).

In order for cancer cells to maintain high rates of proliferation they must produce and utilize large amounts of nutrient, specifically glucose, efficiently. Healthy, normal cells coordinate with several regulatory genes, specifically tumor-suppressor genes and oncogenes, in a highly orchestrated series of pathways in order to proliferate properly and maintain genetic stability. Tumor-suppressor genes exist to halt the cell cycle and/or promote apoptosis, or programmed cell death, whereas oncogenes help regulate cell growth and cell differentiation (Fearon & Bommer, 2008). As a healthy cell develops mutations to either of these gene types, the cell loses its ability to repair mistakes in DNA and prevent abnormalities along the development of the cell cycle. Through a series of simultaneous activation and inactivation of these oncogenes and tumor-suppressor genes, normal cells can divide (Levine & Puzio-Kuter, 2010). It is inherent that cells require

multiple mutations, or "hits," to cause a change in the phenotype of the cell. The dysregulations of these genes due to the mutations of a gene itself, or by hereditary/environmental influences is referred to as the "multiple hit theory" (Ashley, 1969).

Cancer Metabolism Dysfunction

As a healthy cell develops a cancerous identity, it is genetically modified to better exist in hypoxic or anaerobic conditions characterized by "accelerated glycolysis and excessive lactate formation...even under fully oxygenated environments" instead of utilizing a more effective pathway of oxidative phosphorylation to yield sufficient ATP. This phenomenon was discovered in 1923 by a German physiologist, Otto Warburg, and was appropriately termed "the Warburg effect" (Warburg et al., 1927). It is noted in current literature that a triad of glycolytic enzymes, specifically hypoxia-inducible transcription factor-1 alpha (HIF-1α), c-Myc, and p53, are heavily involved in why/how cancerous cells favor a glycolytic flux (Yeung, Pan, & Lee, 2008).

Dysregulation of Glycolytic Factors

Transcription factor HIF-1α, a proto-oncogene, responds to low oxygen microenvironments by activating transcription genes for glucose and lactate transporters (Semenza, 2010). Pathways involved in activating HIF-1α include Ras/Raf/MAPK and PI3-K/Akt pathways (Minet, Michel, Mottet, Raes, & Michiels, 2001). Genes activated by HIF-1α gain of function (GOF) or overexpression include pyruvate dehydrogenase kinase-1 (PDHK-1) and glucose transporter GLUT-1. GLUT-1 has a high affinity for glucose and plays a significant role in tissues that are highly dependent on glucose for energy (Marín-Hernández et al., 2006). PDHK-1 donates a phosphate group to pyruvate

dehydrogenase, thus phosphorylating and inactivating pyruvate dehydrogenase (PDH) (Brooks, 2009). The inactivation of PDH limits the amount of pyruvate that is shuttled to mitochondria and oxidized to acetyl-CoA and instead, further favors shuttling to the glycolytic flux via gluconeogenesis, the synthesis of glucose from non-carbohydrate carbon substances. In short, lactate can be reutilized for ATP synthesis by converting it back into glucose (Semenza, 2007). In addition to PDHK-1 and GLUT-1, another key enzyme that is overexpressed via HIF-1α mediated downstream regulation is lactate dehydrogenase isoform A (LDHA), which favors the reduction of pyruvate to lactate while also yielding a necessary substrate, NAD^+, under highly glycolytic environments (Firth et al., 1995; McCleland et al., 2012).

As with HIF-1α, c-Myc gene expression is also mediated through Ras/Raf/MAPK and PI3-K/AKT signaling pathways (Cairns, Harris, & Mak, 2011). c-Myc overexpression, another major oncogenic target of cancer metabolism, is also partly responsible for many of these carcinogenic shifts. In normal cells, c-Myc is very highly regulated in order to insure its efficiency in positively regulating the expression of numerous genes involved with cellular proliferation and metabolism. In cancer cells, oncogenes including c-Myc experience a "gain of function" (GOF) in which the oncogene ignores signals that stop cell growth by inducing apoptosis and instead accelerate tumor development (Pelengaris, Khan, & Evan, 2002). c-Myc GOF accelerates the level of expression of genes such as GLUT-1 and LDHA (Shim et al., 1997). Similar to HIF-1α, which also overexpresses GLUT-1 and LDHA, c-Myc induced overexpression of GLUT-1 results in an increased amount of glucose that is shuttled into the tumor microenvironment, allowing for increased glucose metabolism and cell energy supply

(Osthus et al., 2000). Because of the HIF-1α mediated activation of PDHK-1, the high levels of lactate produced as a result of accelerated glucose influx are not dispelled into mitochondria to be oxidized to acetyl-CoA, but instead are converted back to glucose via the Krebs cycle (De Saedeleer et al., 2012; Shim et al., 1997).

p53, a tumor suppressor gene, regularly functions to shift away from the glycolytic flux and promote oxidative phosphorylation by repressing the expression of glucose transporters GLUT-1 and GLUT-4 while also upregulating mitochondrion proteins, which would further promote oxidative phosphorylation (Safdar et al., 2015). In addition to regulating protein expression critical to regulating normal cell metabolism, p53 modulates apoptosis in the presence of low glucose levels. Tumor suppressor genes experience a loss of function (LOF) where mutations to the gene prevent its role in regulating mechanisms (Vogelstein & Kinzler, 2004). As a result, in tumorigenesis, p53 loses its ability to repress expression of glucose transporters, which facilitates a highly glycolytic environment. In highly glycolytic environments, p53 ceases to activate apoptosis as well (Bensaad et al., 2006).

Lactate Mediated Angiogenesis

In addition to these metabolic enzymes necessary for carcinogenesis, a group of proteins that are highly expressed by HIF-1α/c-Myc GOF and p53 LOF are monocarboxylate transport proteins (MCT). MCT's are instrumental in facilitating lactate extrusion from cancer cells to the tumor microenvironment and vice versa, and their expression is heavily upregulated upon oncogenic GOF/tumor suppressor gene LOF. Whereas oncogenic dysfunction leads to upregulation of MCT4's which primarily facilitate lactate extrusion from a cell, tumor-suppressor gene dysfunction leads to

upregulation of MCT1's, which primarily facilitate lactate influx into a cell (Boidot et al., 2012; Ullah et al., 2006). This step of lactagenisis is key as lactate extrusion stimulates vascular endothelial growth factor (VEGF) expression leading to increased rates of angiogenesis, the development of new blood vessels, and respective tumor growth (Sonveaux et al., 2012). With increased vasculature to the tumor, there is an increased influx of glucose and other metabolites useful for cell growth and proliferation into the tumor as well as the subsequent flush of excess metabolites, including lactate, from the environment involved with the tumor. HIF-1α and c-Myc oncogenes as well as p53 tumor-suppressor gene all seem to behave geometrically in that their respective gains and losses of function all coincide with another to contribute directly to the Warburg effect; the high affinity of carcinogenic cells to convert glucose to pyruvate even in the presence of adequate oxygen content (Warburg et al., 1927). Patients who express high levels of any combination of these genes have significantly increased mortality rates which is likely due to an increased aggressiveness of their cancer (Brizel et al., 2001; Kunkel et al., 2003; Unruh et al., 2003; Younes et al., 1997). Collectively, as a result of HIF-1α, c-Myc GOF, and p53 LOF, respective up/downregulation of PDHK-1, PDH, LDHA, glucose transporters, and MCT's harmoniously interact with each other to favor a glycolytic flux by increasing glucose concentration in the tumor microenvironment while also preventing lactate oxidation to pyruvate, as well as facilitating proper extrusion of lactate from cancer cells into the tumor microenvironment. A result of this carefully coordinated production, accumulation, and release of lactate is the stimulation of angiogenesis and tumor growth via VEGF overexpression.

Lactate has historically been regarded as a waste product of energy metabolism. However, it has become increasingly evident that lactate is a key player in cancer prognosis and aggressiveness not because of its role in pH acidification of cancer cells and the tumor microenvironment but because of its role as a signaling molecule involved with different mechanisms sustaining cancer cell survival, proliferation, and metastasis (Gottfried, Kreutz, & Mackensen, 2012). Cancer-mediated lactagenesis, the generation or production of lactate through the initiation of gene mutations, behaves in a circular pattern leading to metabolic reprogramming and the increased accumulation and disposal of lactate in the tumor microenvironment. The coordination of HIF-1α/c-Myc GOF and p53 LOF results in an increase in glycolytic enzyme expression and activity and therefore an influx of glucose uptake by the tumor microenvironment (Bensaad et al., 2006; Brooks, 2009; Shim et al., 1997). Because lactate oxidation to pyruvate is repressed in tumor cells, this increased glucose uptake is characterized by increased lactate production and accumulation through a catabolic process known as anaerobic glycolysis. Through the catabolism of a glucose molecule, ultimately, two molecules of lactate are produced while also yielding an oxidized dinucleotide, NAD^+, essential to sustaining rapid rates of glycolysis and redox homeostasis, which is what is seen in strenuous exercise (Dang & Semenza, 1999). It is important that NAD^+ is produced intracellularly as these dinucleotides do not diffuse across cellular membranes easily. Cancer metabolism is inherently similar to that of Type IIB/IIX skeletal muscle fiber in that high rates of glycolysis continue to yield high rates of NAD^+, which is then utilized to continue higher rates of glycolysis and its accompanied lactate production (Semenza et al., 1996). With the onset of lactate production, upregulation of MCT's are stimulated thus facilitating an

increase in lactate extrusion as well as lactate-mediated angiogenesis through VEGF. As more blood, and glucose, cycle through the tumor microenvironment, more glucose is metabolized, therefore continuing the cycle of cancer-mediated lactagenesis while also providing a favorable milieu for tumor cell growth and survival.

Lactate and Exercise

As the rate of ATP demand exceed ability to provide that ATP aerobically, energy production switches from primarily oxidative phosphorylation to primarily aerobic and anaerobic glycolysis. It is well established that oxidative phosphorylation is far more efficient in yielding the necessary amount of energy, approximately 36 molecules of adenosine triphosphate (ATP) per molecule of glucose, for muscular contraction and movement during exercise, but the issue is that oxidative phosphorylation demands a much more extensive time component**.** Anaerobic glycolysis can only yield up to two molecules of ATP, but in just a fraction of the time. This energy molecule, ATP, is required for a number of metabolic processes, including skeletal muscle contraction, and it is this swift switch to anaerobic metabolism that leads to an abrupt increase in lactate and its paired hydrogen ion. Lactate accumulation in skeletal muscle is traditionally associated with a burning sensation related to several theories of muscle fatigue and limitations of exercise performance. It is well known that lactate and its associated hydrogen ion contribute to impairments in muscle contractility. Upon the onset of high intensity exercise, the energy demand for myosin-actin crossbridge cycling and increased myosin adenosine triphosphatase (ATPase) activity must be met through anaerobic metabolic processes because aerobic metabolic processes require too much time. When there is a high demand for ATP paired with an oxygen deficiency, pyruvate instead of

being shuttled to slow oxidative muscle fiber that contain high densities of mitochondria via MCT's, is converted to lactate to yield an oxidized NAD^+ to promote energy production via a glycolytic flux. Several frog, rodent, and human studies have shown that intracellular hydrogen ions derived from lactate production often disassociate and impair production of metabolic proteins such as phosphocreatine and phosphofructokinase, necessary for energy production in an oxygen-lacking environment, known as intramuscular acidosis (Sahlin, 1986). Additionally, Donaldson, Hermansen, & Bolles (1978) and Westerblad, Bruton, & Lännergren (1997) conducted individual studies in which rat muscles predominant in fast twitch fibers were connected to a force transducer and stimulated to induce tetanic contractions and the onset of fatigue. Maximal forces were measured at each stimulation and researchers found that force production as well as shortening velocity both decreased as intramuscular pH levels decreased. Intramuscular acidosis, according to Balog & Fitts (2001), has been shown to affect the rates of sarcoendoplasmic reticulum calcium ATPase (SERCA) Ca^{2+} release and reuptake to/from crossbridge binding sites, thus limiting the rate of crossbridge formation during exercise (Favero, Zable, Bowman, Thompson, & Abramson, 1995). Even further, it has been shown that hydrogen protons can bind to myosin ATPase binding sites themselves on myosin heavy chain muscle fiber(s), further impairing the rate of crossbridge formation and cycling, thus reducing force output and muscle fiber shortening velocity.

Blood Lactate Parameters During Exercise

Lactate production, accumulation, and disposal during exercise has distinct characteristics during exercise that have been quantified. Lactate as a byproduct of carbohydrate utilization for energy production is always being produced and extruded

into vasculature and thus, a resting level of blood lactate accumulation exists at all times typically ranging from 0.9 to 1.5 mmol/L (Tesch, Sjodin, & Karlsson, 1978). It is a heavily recycled metabolite, reutilized by tissue in the liver, kidneys, heart, brain, and adjacent skeletal muscle and is extruded into the vasculature at higher rates during exercise, depending on the glycolytic capacity of the working muscles (Van Hall, 2010). A valid and reliable method of measuring lactate extrusion into the vasculature during exercise is the use of blood lactate analyzers, similar to diabetic blood glucose meters. Depending on the type of muscle and the exercise intensity, as well as whether the individual is trained or untrained, high levels of blood lactate accumulation can occur sooner into an exercise bout rather than later due to the aerobic capacity of different muscle fiber. Type IIB/X muscle fibers have less mitochondrial density and thus, a lower aerobic capacity to shuttle pyruvate into mitochondria to be utilized via oxidative phosphorylation and so the onset of lactate accumulation would occur faster (Adeva-Andany et al., 2014). As exercise intensity increases, the metabolic demand for energy or ATP increases. ATP synthesis is a direct product of anaerobic and aerobic glycolysis, and lactate is formed amongst many other metabolites. As the body's metabolic demand increases, cardiovascular activity increases as does the rate at which blood flows throughout the vasculature. The amount of lactate, and other metabolites, produced and diffused into the blood stream increases. This increased value of blood lactate can help clinicians or even coaches determine at what intensity or metabolic equivalent (MET) the individual or athlete is working by establishing the individuals' Lactate Threshold 1 (LT1), or the moment at which there is a sustained increase in blood lactate above resting levels, and Lactate Threshold 2 (LT2), which is the upper limit of blood lactate

accumulation indicating an equilibrium between lactate production and lactate elimination. Determining an individual's onset of blood lactate accumulation (OBLA), which refers to the exercise intensity, or METS, that blood lactate begins to exponentially increase, can also help a professional determine what specific types of exercise modalities to incorporate into their programming. (Adeva-Andany et al., 2014; Stegmann, Kindermann, & Schnabel, 1981).

Blood Lactate Parameter Adaptation
Response to Exercise

As it is important to understand how and why populations reach different blood lactate parameters at different percentages of their maximal oxygen uptake, it is also important to understand how mechanisms of lactate production, accumulation, and disposal adapt to exercise training. At higher intensities or METS, the capacity of which ATP is derived to sustain the intensity of exercise shifts from an aerobic capacity to an anaerobic capacity (Mougios, 2006). Typically, glucose is broken down into ATP and pyruvate via glycolysis; the pyruvate produced is shuttled into adjacent non-exercising tissue such as the heart, liver, kidneys, brain, and non-working skeletal muscle where pyruvate is degraded further for energy production via oxidative phosphorylation (Essen, Pernow, Gollnick, & Saltin, 1975; Naughton & Hellerstein, 1973). The rate at which an individual consumes and utilizes oxygen can determine at what intensities there might be a shift from aerobic to anaerobic metabolism for energy production during exercise. This implies that as exercise intensity increases, the rate of which lactate accumulates in the blood succeeds the rate at which blood lactate is resorbed and that the demand for ATP cannot be met through oxidative phosphorylation. With exercise training there is an increase in ventilatory threshold, the reflection of anaerobiosis and lactate accumulation

in catabolizing tissue, and the ability to recycle said lactate for oxidative phosphorylation (Bassett & Howley, 2000).

The present study utilizes METS, the amount of oxygen consumed/utilized multiplied by the resting value, 3.5 ml/kg/min, to measure improvement of exercise tolerance and buffering of lactate accumulation with increasing intensity of exercise. With the intervention of exercise, as is seen with apparently healthy populations, an individuals' capacity to consume oxygen with the goal of producing energy through aerobic means would improve. With an exercise-mediated improved ability to consume and utilizing oxygen, an individual would achieve OBLA at higher intensities, and METS, of exercise due to subsequent improvements in buffering lactate through oxidative mechanisms (Jette, Sidney, & Blümchen, 1990). The question with cancer populations, who express dysfunctions in metabolic behavior, is if they would respond similarly to exercise in terms of lactate buffering at higher intensities or METS during an exercise bout to volitional fatigue.

Endurance Training

A change in lactate parameters of resting, LT1 and LT2, OBLA, and peak blood lactate accumulation levels would signify a physiological adaptation in how an individual efficiently produces, accumulates, and disposes of lactate from tissue actively utilizing energy. Research has shown endurance training induced increases in HIF-1α oncogene expression (Abe et al., 2015) as well as mRNA expression and translocation of GLUT-1 & 4 glucose transporters, facilitating high rates of glucose uptake in skeletal muscle as well as increased insulin sensitivity in diabetic patients (Pouysségur, Dayan, & Mazure, 2006; Wojtaszewski, Hansen, Kiens, & Richter, 1997). In addition to glucose

transporters, endurance training also increases MCT1 & MCT4 content allowing for increased extrusion and cell-to-cell uptake of lactate during exercise according to a rat study conducted by Aveseh, Nikooie, & Aminaie (2015). This same study also monitored LDH enzymes and showed increased expression in LDH-B enzymes and decreased expression in LDH-A enzymes, facilitating lactate to pyruvate oxidation for further oxidative phosphorylation while also preventing pyruvate reduction to lactate, thus buffering the accumulation of lactate extruded into the vasculature (Bishop, Edge, Thomas, & Mercier, 2008). p53 tumor-suppresor genes were also monitored in a study by Safdar et al., (2015). The study reported a reduction in mitochondrial DNA mutation and increased rates of oxidative phosphorylation with the intervention of endurance exercise in aging mice. It is also noted that exercise-induced lactate shuttles are 'sensed' by the proto-oncogenic Myc network, thus stimulating the production of glucose transporters and glycolytic enzymes necessary for improved lactate handling during exercise. As exercise intensity increases, c-Myc oncogenes sense the increase in redox-modulating metabolites such as NAD^+ and lactate handling in order to promote the upregulation of necessary glycolytic intermediates, allowing for increased lactate production, accumulation, and disposal (Gohil & Brooks, 2012).

Resistance Training

Far less information exists on the implications of resistance exercise training and oncogenic/tumor-suppressor gene regulation as well as its relationship with glycolytic enzyme expression. Some resistance training studies in humans have shown upregulation of glucose transporter isoforms 1 and 4 as well as lactate dehydrogenase enzymes (Rodrigues et al., 2010). Along with glucose transporters, monocarboxylate transport

proteins also seem to be upregulated with resistance training. A study investigating the relationship between MCT 1 and 4 protein expression and resistance training in diabetic patients reported that MCT content is typically reduced in diabetic patients, however this reduction can be attenuated with resistance training (Juel et al., 2004). Resistance training did, although, increase MCT content in skeletal muscle fiber of healthy adult males.

Lactate and Cancer Therapies

Lactagenic intermediates and symporters have also been shown to increase during bouts of chemotherapy and radiation, suggesting the increased mobilization of lactate during typical cancer therapies. Several studies show the increased regulation of LDHA and B isoforms as well as both MCT1 and 4 isoforms, facilitating the production, accumulation, and disposal of lactate for carcinogenic proliferation (McCleland et al., 2012; McCleland et al., 2013). The targets of these chemotherapeutic drugs and radiation are to eliminate the dysregulation of HIF1α/c-Myc oncogenic and p53 tumor-suppressor gene expression. A combination of both endurance and resistance exercise modalities seems to modulate HIF-1α oncogenic and p53 tumor suppressor gene expression as well as glycolytic enzymes and transport proteins involved with lactate production, accumulation, and disposal. An increase in lactate production and extrusion also stimulates c-Myc expression, further promoting lactate production through the glycolytic flux. Through HIF-1α, c-Myc, and p53 mediated upregulation of glucose transporters and glycolytic intermediates shifting metabolic regulation towards lactagenesis, it may be plausible to infer improved lactate handling in both apparently healthy and cancer populations at similar METS through the application of endurance and/or resistance exercise. Theoretically, lactate mediated VEGF expression could also deter vascular

growth from the tumor microenvironment and instead allocate blood flow to working skeletal muscle and other tissues. In addition, exercise mediated regulation of glycolytic proteins and its subsequent regulation of lactate production, accumulation, and disposal may shunt lactate as a metabolic regulator in tumorigenesis.

Resting Lactate in Cancer Survivors

It is important to note in a study conducted by Brizel et al., (2001) that lactate concentrations in head and neck cancer survivors were up to 40% higher than that of an apparently healthy population. Because the process of lactagenesis and its accompanied glycolytic intermediates are so heavily upregulated, the facilitation of glucose into the tumor and its subsequent lactate production, accumulation, and extrusion is enhanced. Resting lactate levels of apparently healthy individuals are typically between 0.9 and 1.5 mmol/L, however can reach up to 40mmol/L in cancer survivors with evidence of metastasis (Tesch et al., 1978; Walenta et al., 1997). Blood is continuously flowing through the human body delivering and collecting metabolites to and from tissues to promote a homeostatic environment. Upon the initiation of exercise, skeletal muscle contraction mediated blood flow increases the rate at which blood flows throughout the vasculature, enabling the vasculature to deliver and collect metabolites at higher rates to meet the energy demands of different tissue (Jorfeldt & Wahren, 1971; Lash, 1996). It is plausible to believe that at least a percentage of lactate that is being produced by tumors is extruded into the vasculature and can be measured. Furthermore, upon the onset of increased exercise-mediated flow, it is plausible to believe that nutrients typically directed to tumors can be redirected to working skeletal muscle and other tissues during exercise, thus depleting the amount of nutrient a tumor would typically receive at rest.

This application of exercise to reduced rates of flow to a tumor may perhaps reduce the rate at which tumors can grow, produce lactate, and stimulate angiogenesis, or even starve the tumor itself.

Conclusion

Tumors, even in the presence of adequate oxygen saturation, prefer anaerobically glycolytic methods of energy production and the subsequent overproduction of lactate. It has been discovered that three genes, HIF-1α, c-Myc, and p53, are largely responsible for the upregulation of glycolytic intermediates including lactate dehydrogenase enzymes, glucose transport proteins, and monocarboxylate transport proteins. Current research in cancer metabolism has effectively characterized the pattern in which carcinogenic cells upregulate said factors, leading to high volumes of glucose entry into the tumor and the subsequent overproduction, accumulation, and disposal of lactate without its oxidation to pyruvate for further oxidative processes.

Lactate continues to be associated with signaling mechanisms for the upregulation of glucose transporters and MCT's necessary for improved lactate handling. As increased rates of lactate exit tissue environments, decreased pH levels stimulate acidosis-induced angiogenesis. This increase in vasculature provides the tumor with more nutrients for growth and proliferation, allowing the tumor to continue along a vicious cycle of glycolysis and lactate production.

High levels of lactate concentrations in cancer survivors is associated with increased risks of metastasis and cancer aggressiveness. It is well established that exercise can attenuate the production of lactate through the upregulation of transport proteins and following oxidative mechanisms necessary for oxidative phosphorylation,

which are typically down regulated in cancer survivors. Through exercise mediated flow and metabolic adaptations to exercise, it may be possible to effectively redirect metabolites away from the tumor microenvironment and towards metabolic processes of active tissue during exercise.

Very few studies have investigated lactate production in cancer survivors and no studies have monitored lactate production before and after an exercise-intervention. The purpose of this study was to assess the effect of exercise training on lactate handling in cancer survivors, and further, to assess any differences between those actively receiving cancer therapies and those not. This study provides much needed knowledge about the effects of an exercise intervention on lactate production, accumulation, and disposal in the cancer population.

CHAPTER III

EFFECTS OF EXERCISE TRAINING ON THE BLOOD LACTATE RESPONSE TO ACUTE EXERCISE IN CANCER SURVIVORS

Abstract

Cancer metabolism, emerging as a clear biomarker of cancer, is characterized by increased levels of lactate production, even in the presence of adequate oxygen saturation. This is a preliminary investigation on blood lactate response in cancer survivors actively undergoing cancer treatment (active) vs cancer survivors not currently going through cancer treatment (inactive). Exercise has been shown to regulate lactate production, accumulation, and disposal in diseased populations, however little is known about the exercise effects on lactate handling in a cancer population. **Purpose:** To determine the effect of a 12-week exercise intervention on lactate parameters in cancer survivors in treatment versus out of treatment during an exercise bout to volitional fatigue. **Methods:** Twenty-one cancer survivors were recruited for a 12-week exercise-based oncology rehabilitation intervention (active n = 7; inactive n = 14). Prior to the 12-week exercise intervention, cardiovascular endurance was assessed using a progressive treadmill protocol to volitional fatigue. Blood lactate accumulation (BLA) was quantified every two minutes during the exercise assessment via finger stick and compared to measures taken during a reassessment treadmill protocol 12 weeks later. **Results:** Resting, Peak, and METS at OBLA values of active and inactive cancer survivors before and after the exercise intervention were not significantly different ($P>0.05$). Furthermore,

Resting, Peak, and METS at OBLA values between active and inactive cancer survivors before and after an exercise intervention were not significantly different ($P>0.05$). However, METS at OBLA in both active and inactive survivors were significantly different before and after a 12-week exercise intervention ($P<0.05$). **Conclusions:** Lack of significance in these findings may indicate similar responses of lactate handling to exercise training in both cancer groups. This finding is supported by a significant increase in METS at OBLA of the total subject population before and after the exercise intervention suggesting an improved ability to buffer lactate at higher intensities during an acute exercise bout to fatigue. **Keywords:** BLOOD LACTATE ACCUMULATION, CANCER SURVIVORS, EXERCISE, METABOLIC EQUIVALENTS, ONSET OF BLOOD LACTATE ACCUMULATION

Introduction

Cancer is defined by abnormal, uncontrolled cell division and its respective spread to other parts of the body through the blood and lymph systems (National Program of Cancer Registries , 2018). As diagnoses of cancer increase, cancer-mediated dysfunction in metabolic behavior and related lactate production continues to emerge as a clear characteristic of cancer; a step along the road of carcinogenesis identified amongst a majority of cancers (Cairns et al., 2011). Normal cells proliferate under a series of heavily regulated pathways and control mechanisms. When normal cells are affected by mutations caused by environmental or hereditary influences, they are no longer governed by genetic regulation but instead divide and proliferate rapidly and abnormally. In order to sustain high rates of cell proliferation and division, cancerous cells fundamentally change their metabolic characteristics in favor of glycolytic pathways even when oxygen is abundant. A byproduct of high rates of glycolytic processes results in the subsequent production of lactate, however this accumulation of lactate is not buffered by successive oxidation to pyruvate in tumors, according to German physiologist Otto Warburg, as it is in apparently healthy cells (Warburg et al., 1927). Instead, through a series of upregulated glycolytic intermediates and transport proteins, lactate is extruded from the tumor microenvironment and continues to act as a stimulatory mechanism resulting in increased rates of angiogenesis via acidosis-induced angiogenesis (Sonveaux et al., 2012). Respective oncogenic and tumor-suppressor gene gain of function and loss of function involved with carcinogenesis resulting in increased rates of lactate production,

accumulation, and disposal are associated with the subsequent development of metastasis and cancer aggressiveness (Kunkel et al., 2003).

Lactic acid, a lactate molecule paired with a hydrogen proton, is produced in excess during prolonged moderate-to-high intensity exercise. As rates of ATP production via oxidative phosphorylation are unable to meet the energy demand of increasing exercise intensity, the human body efficiently switches to glycolytic-based energy production, yielding ATP far quicker than that of oxidative phosphorylation. In other words, as catabolic demands exceed the rate of maximal oxygen consumption, energy metabolism switches from oxidative phosphorylation to glycolytic methods of energy production. Physiologists have quantified this metabolic switch during maximal exercise and defined it as an individual's Lactate or Anaerobic Threshold, where rates of oxygen consumption and utilization cannot meet the demand for ATP production resulting in the successive overproduction of lactate and decreased pH levels.

As we know that lactate is an important metabolic indicator for cancer metabolism as well as evidence of metastasis and tumor aggressiveness, determining the response of blood lactate during progressive exercise bouts in a cancer population may provide some insight into the effects of exercise on cancer development, progression, and metastasis. Both endurance and resistance exercise modalities have been shown to aid in gene regulation as it relates to lactate handling. The purpose of this study was to investigate the blood lactate accumulation response to a progressive exercise bout to volitional fatigue before and after a 12-week exercise-based oncology rehabilitation intervention between cancer survivors currently undergoing treatment and cancer survivors not currently undergoing treatment.

Methodology

Experimental Design

The purpose of this study was to investigate blood lactate accumulation response to a progressive exercise bout to volitional fatigue before and after a 12-week exercise-based oncology rehabilitation intervention between cancer survivors currently undergoing treatment and cancer survivors not currently undergoing treatment. Cancer survivors were divided into two groups: those actively receiving chemotherapy or radiation and those not currently receiving chemotherapy or radiation. Participants were screened through an inclusive screening process of physiological and psychological parameters, including blood lactate accumulation (BLA) during a progressive exercise bout to volitional fatigue. BLA response in mmol/L was measured every two minutes during the UNCCRI Treadmill Protocol, a graded exercise assessment, using a Nova Biomedical Lactate Plus Meter along with Lactate Plus Meter test strips and safety lancets. BLA response was measured during the initial assessment and again after a 12-week exercise intervention, and the results were compared.

Subjects

Twenty-one cancer survivors were referred by their oncologists or primary care physician and recruited to participate in a 12-week exercise-based rehabilitation intervention study at the University of Northern Colorado Cancer Rehabilitation Institute (UNCCRI). Cancer survivors recruited for this study underwent a comprehensive screening process, assessing pertinent cancer related information and treatment history, additional medical information, family history and other disease concerns, and current

physical activity levels. Participants were divided into two groups: active cancer survivors, or survivors currently undergoing chemotherapy or radiation, (n=7) and non-active cancer survivors, survivors not currently undergoing chemotherapy or radiation (n=14). All participants in this study were volunteers through the University of Northern Colorado Cancer Rehabilitation Institute (UNCCRI) at the University of Northern Colorado located in Greeley, CO. Inclusion criteria for this study included both male and female cancer survivors with or without metastasis regardless of treatment status and physical activity level.

This human subjects study was reviewed and approved by the Institutional Review Board at the University of Northern Colorado. Before agreeing to this study, participants were given a UNCCRI research participation informed consent form to review and sign, were informed of the specific study and all risks associated with the study, and verbally consented to volunteering for the study. All participants were cleared by their oncologist and/or primary care physician to participate in an exercise-based rehabilitation program.

University of Northern Colorado
Cancer Rehabilitation Institute
12 Week Exercise Based
Rehabilitation

Prior to the study, all participants performed an initial assessment supervised by staff exercise physiologists. This assessment quantified pulmonary function, muscular strength, and cardiovascular fitness. Pulmonary function was measured by staff Cancer Exercise Specialists (CES) and assessed using a MIR Spirolab III pulmonary function device (MIR Group USA Head Office, New Berlin, Wisconsin) to measure Forced Vital Capacity (FVC) and Force Expiratory Volume (FEV). Muscular strength, defined by a

series of estimated 1 repetition maximum tests in both lower and upper extremity exercises, was measured by staff CES and assessed utilizing Selectorized Cybex Eagle strength training equipment (Owatanna, Minnesota). Cardiovascular endurance was assessed utilizing the UNCCRI Treadmill Protocol, a valid and reliable graded Vo_{2peak} exercise assessment where speed and/or grade are increased every minute, on a treadmill (Shackelford, Brown, Peterson, Schaffer, & Hayward, (in press)). Lactate accumulation was measured during the UNCCRI treadmill protocol using a Nova Biomedical Lactate Plus Meter along with Lactate Plus Test Strips and Safety Lancets (Waltham, Massachusetts).

Upon completion of the initial assessment, CES use the comprised data to generate an individualized 12-week exercise-based oncology rehabilitation intervention consisting of 20 minutes of cardiovascular endurance exercise, 30 minutes of resistance training, and 10 minutes of balance and flexibility training. The intervention follows guidelines defined by the Phase Training protocol for oncology-based exercise rehabilitation, developed within at UNCCRI (Brown JM, Shackelford DYK, Cress M, Hayward R. Evaluation of an Exercise-Based Phase Program as Part of a Standard Care Model for Cancer Survivors. Translational Journal of the American College of Sports Medicine (in press)). Active survivors, defined here as those currently undergoing chemotherapy or radiation, entered the phase training protocol in Phase 1, whereas inactive survivors, defined here as those not currently undergoing chemotherapy or radiation, have recently received surgical intervention, or hormonal/immunotherapy, entered the phase training protocol in Phase 2. The phase training protocol largely refers to a strict range of exercise intensities for an individual participant throughout the 12-

week exercise intervention. Cardiovascular intensities are based on a percentage of heart rate reserve (HRR), which is calculated using the Karvonen formula. Phase 1 participants exercise at low to moderate intensity, or 30-45% of the individual's HRR according to ACSM guidelines, in order to efficiently preserve health status and combat toxicities of treatment. Phase 2 participants are eligible to exercise at a moderate intensity, defined as 40-60% of their individual HRR according to ACSM guidelines, with the purpose of building functional foundation of movement structured around the individual while also eliciting chronic adaptations to exercise (American, 2013). After every 12 weeks, participants are reassessed through all physiological parameters assessed during the initial assessment and are given a new 12-week exercise progression through their next appropriate phase.

Lactate Measurement

Lactate samples were collected before, after, and throughout the UNCCRI treadmill protocol during a participant's initial assessment and at their respective reassessment 12 weeks thereafter. Lactate samples were collected on the ring finger of the right hand unless lymph nodes have been removed from the right arm. Regardless of the presence of lymphedema, if lymph nodes had been removed from the right arm, lactate samples were collected from the ring finger of the left hand. Participants were given a rechargeable hand warmer to stimulate increased blood flow to the fingertips in order to prevent sticking the finger several times. After the hand was warmed for two to three minutes, the hand warmer was turned off and a sterile Curad Alcohol Prep Pad (Mundelein, Illinois) was used to wipe and clean the surface of the finger. After waiting approximately 45 seconds for the alcohol to dry, the finger was stuck with a Nova

Biomedical Lactate Plus Safety Lancet (Waltham, Massachusetts). With both thumbs, the researcher then applied upward pressure from the bottom of the finger to the fingertip to allow a droplet of blood to form. The droplet of blood was then wiped away with a sterile Covidien Curity 2" x 2" gauze pad (Mansfield, Massachusetts) in order to wipe away any residual alcohol left on the finger, which may skew the lactate measure or cause an error. After a second droplet of blood was formed, the droplet was collected and analyzed for 15 seconds via a Lactate Plus Meter and Lactate Plus Test Strips (Mansfield, Massachusetts) and recorded on the "Resting Measure" area of a UNCCRI lactate accumulation protocol chart in mmol/L. Upon collecting samples, researchers held the analyzer slightly horizontal as to prevent blood from touching the bottom surface of Lactate Plus Test Strip, as this may result in an error. Prior to the initiation of the UNCCRI treadmill protocol, participants were asked to recall a food log of the present day and were instructed on proper procedure for lactate sampling during an exercise assessment to volitional fatigue; participants were instructed to, while walking or running, place their hand palm side up on the handrail of the treadmill while the researcher collected a droplet of blood.

Researchers collected samples approximately every two minutes utilizing the same upward-pressure technique mentioned above. Lactate accumulation measures were recorded on the same protocol chart developed by UNCCRI along with a Rating of Perceived Exertion (RPE), which was quantified every three minutes by the participant. So as not to impede with the efficacy of the treadmill protocol and its estimation of Vo_{2peak}, lactate accumulation measurements were halted at an RPE of approximately 6 or 7. Lactate accumulation was measured immediately after the termination of the exercise

assessment and then again after an individualized cool down phase. After a final cool-down, lactate accumulation measurement was taken, another Curad alcohol prep pad was used to wipe away residual blood on the finger and a bandage was applied to prevent blood from contacting any external surfaces. Lactate Plus Saftey Lancets are disposed of in a biohazard sharps only container. The blood lactate accumulation data collection form appears in Figure 1.

Client Number: **Date:** **Initial or Reassessment:**

Cancer Type:

Cancer Stage:

In Treatment (Yes or No):

- Treatment Type:

Tumor (Active or Removed):

Did subject use handrails?

Did subject start to run?

Age:

Height:

Weight:

Body Fat %:

Stress Score:

24 Hour Food Recall:

Lactate Concentration (Approx. every 2 min)	**RPE (Most recent at time of draw)**
Resting	
Draw 1	
Draw 2	
Draw 3	
Draw 4	
Draw 5	
Draw 6	
Draw 7	
Draw 8	
Draw 9	
Draw 10	
Termination Draw	
Cool-Down Draw	

Notes/Medications:

Figure 1: The UNCCRI Blood Lactate Accumulation Data Collection Form

Metabolic Equivalents

Metabolic Equivalents (METS) were used to evaluate changes in lactate parameters and lactate handling during a progressive exercise bout to volitional fatigue before and after a 12-week exercise-based oncology rehabilitation intervention. UNCCRI has compiled an assessment manual containing normative values for all physiological and psychological parameters assessed throughout the exercise intervention to better identify meaningful adaptations to exercise training. METS are used to estimate the energy expenditure of many different physical activities, where one MET is equal to 3.5 milliliters of oxygen consumed per kilogram of bodyweight per minute. METS were calculated at each stage of the UNCCRI treadmill protocol for both walking and running aspects of the assessment, according to the UNCCRI assessment manual. OBLA is defined as the intensity of exercise at which lactate begins to accumulate exponentially and was quantified objectively. If OBLA values ≥4.0 mmol/L, were not obvious at a particular stage of the UNCCRI treadmill protocol, either the stage of termination or the stage before were utilized to calculate METS at OBLA.

Statistical Analysis

Means and standard deviations were calculated for Resting, Peak, and METS at OBLA for the groups combined as well as active and inactive cancer survivors before and after a 12-week oncology-based exercise rehabilitation intervention. A paired two-tailed t-test analysis was used to test for significance between variables before and after a 12-week oncology-based exercise rehabilitation intervention. An alpha level of less than 0.05 was considered significant.

Results

Table 1 summarizes values before and after a 12-week exercise-based oncology rehabilitation intervention. Twenty-one participants comprised of 12 females and 9 males volunteered for this study. Of the 21 total participants, 7 were classified as active cancer survivors: those actively receiving chemotherapy or radiation treatment. Fourteen were classified as inactive cancer survivors: those not actively receiving chemotherapy or radiation treatment. All participants were able to safely complete all protocols described for the present study without any adverse events.

Table 1

Subject Demographics

	Active	Inactive
Total n	7	14
Gender		
Females	3	9
Males	4	5
Age		
≥60	5	5
≤60	2	9
Treatment Types		
Chemotherapy	4	5
Radiation	1	4
Both	2	2
None	-	3
Stage (pre)	7.57±2.92	8.86±2.26
Stage (post)	9.57±2.66	9.71±2.76
Resting (mmol/L) (pre)	2.44±0.92	1.84±0.74
Peak (mmol/L) (pre)	6.2±2.67	6.26±2.91
Resting (mmol/L) (post)	1.98±0.62	1.62±0.66
Peak (mmol/L) (post)	7.96±1.75	7.88±3.79
METS at OBLA (ml/kg/min) (pre)	5.96±1.39	6.77±1.59
METS at OBLA (ml/kg/min) (post)	7.36±1.80	7.73±1.44

METS = Metabolic Equivalents; OBLA = Onset of Blood Lactate Accumulation. Data for Pre- and Post- measurements are displayed as Mean ± SD. Significance levels for Pre- to Post- measurements are displayed in Post- data sets for Active and Inactive survivors respectively. *Denotes significance, $P<0.05$

Resting Lactate Accumulation

Mean resting lactate accumulation (RLA) for active cancer survivors decreased from 2.44 ± 0.92 to 1.98 ± 0.62 mmol/L (p = 0.33) after a 12-week exercise intervention (Figure 1). Mean RLA for inactive cancer survivors also decreased from 1.84 ± 0.74mmol/L to 1.62 ± 0.66mmol/L (p = 0.44) after a 12-week exercise-based oncology rehabilitation intervention (Figure 2). RLA between active and inactive cancer survivors lacked statistical significance before and after an exercise intervention (P = 0.19, P = 0.26 respectively).

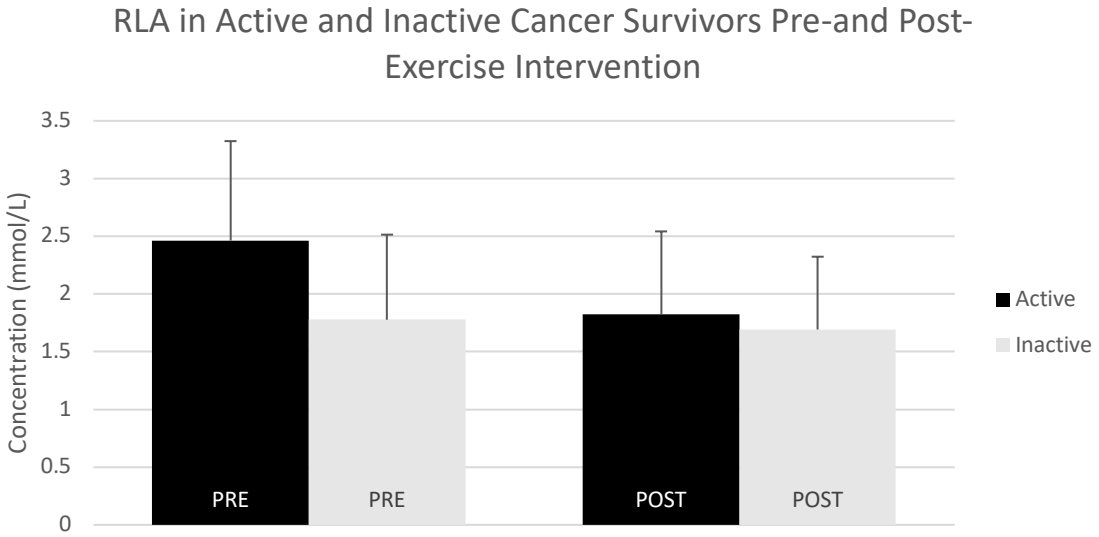

Figure 2: Resting Lactate Accumulation in Cancer Survivors
Pre (Left) and Post (Right) display mean resting lactate accumulation concentrations (mmol/L) with standard deviations in active (n=7) and inactive (n=14) cancer survivors before and after a 12-week exercise-based oncology rehabilitation intervention. No significant differences were observed.

Peak Lactate Accumulation

Mean peak lactate accumulation (PLA) for active cancer survivors increased from 6.20 ± 2.67mmol/L to 7.96 ± 1.75mmol/L (p = 0.21) after a 12-week exercise-based oncology rehabilitation intervention (Figure 3). Mean PLA for inactive cancer survivors also increased from 6.26 ± 2.91mmol/L to 7.88 ± 3.79mmol/L (p = 0.28) after a 12-week exercise-based oncology rehabilitation intervention (Figure 4). PLA between active and inactive cancer survivors lacked statistical significance before and after an exercise intervention (P = 0.97, P = 0.84 respectively).

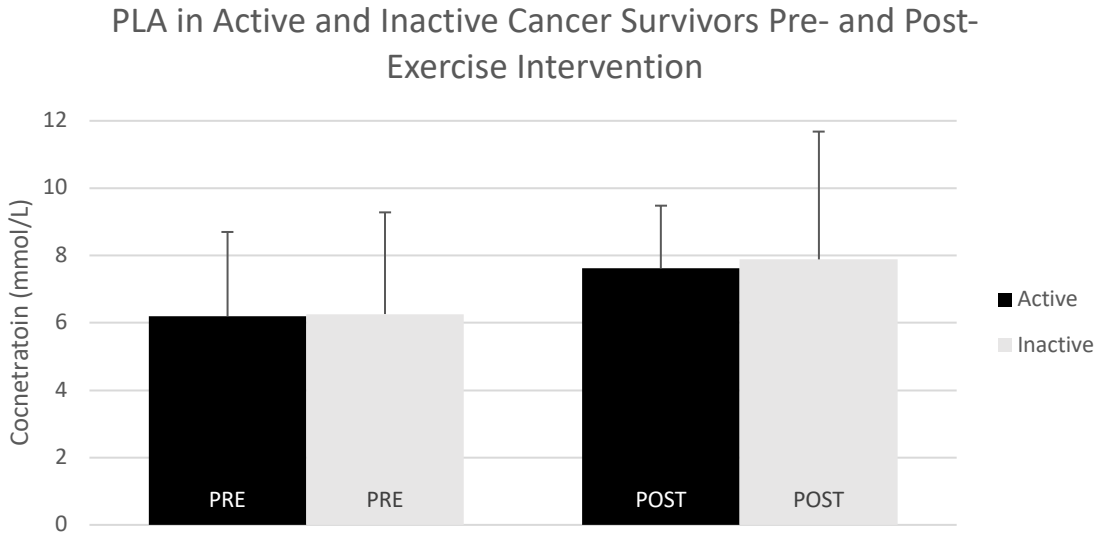

Figure 3: Peak Lactate Accumulation in Cancer Survivors
Pre (Left) and Post (Right) display mean peak lactate accumulation concentrations (mmol/L) with standard deviations in active (n=7) and inactive (n=14) cancer survivors before and after a 12-week exercise-based oncology rehabilitation intervention. No significant differences were observed.

Metabolic Equivalents at Onset of Blood Lactate Accumulation

Mean metabolic equivalent at the stage of OBLA (METOBLA) of active cancer survivors increased from 5.96 ± 1.39 O_2/kg/min to 7.36 ± 1.80 O_2/kg/min (p = 0.14) after a 12-week exercise-based oncology rehabilitation intervention (Figure 5). Mean METOBLA of inactive cancer survivors increased from 6.77 ±1.59 O_2/kg/min to 7.73 ± 1.44 O_2/kg/min (p = 0.19) after a 12-week exercise-based oncology rehabilitation intervention (Figure 6). RLA between active and inactive cancer survivors lacked significance before and after an exercise intervention (p = 0.48, p = 0.77 respectively). However, when comparing the effects of a 12-week exercise intervention on METOBLA in the overall sample size (n=21), METOBLA increased from 6.29 ± 1.58 to 7.51 ± 1.74 (p = 0.04) (Figure 7). Lack of significance in active and inactive cancer survivor groups individually may be due to insufficient sample size.

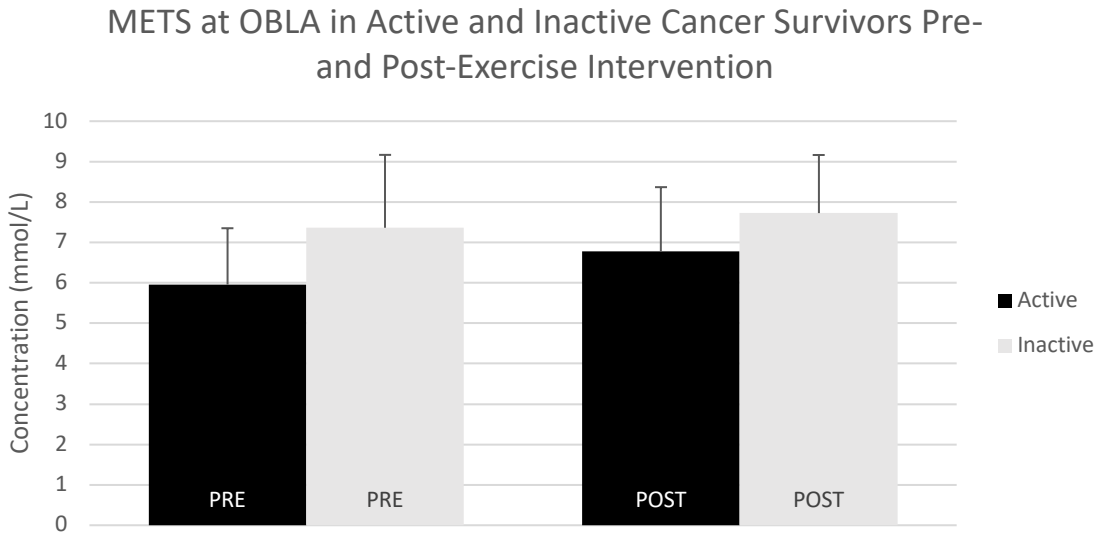

Figure 4: METS at OBLA in Cancer Survivors
Pre (Left) and Post (Right) display mean Metabolic Equivalents (METS) (ml/kg/min) at Onset of Blood Lactate (OBLA) with standard deviations in active (n=7) and inactive (n=14) cancer survivors before and after a 12-week exercise-based oncology rehabilitation intervention. No significant differences were observed.

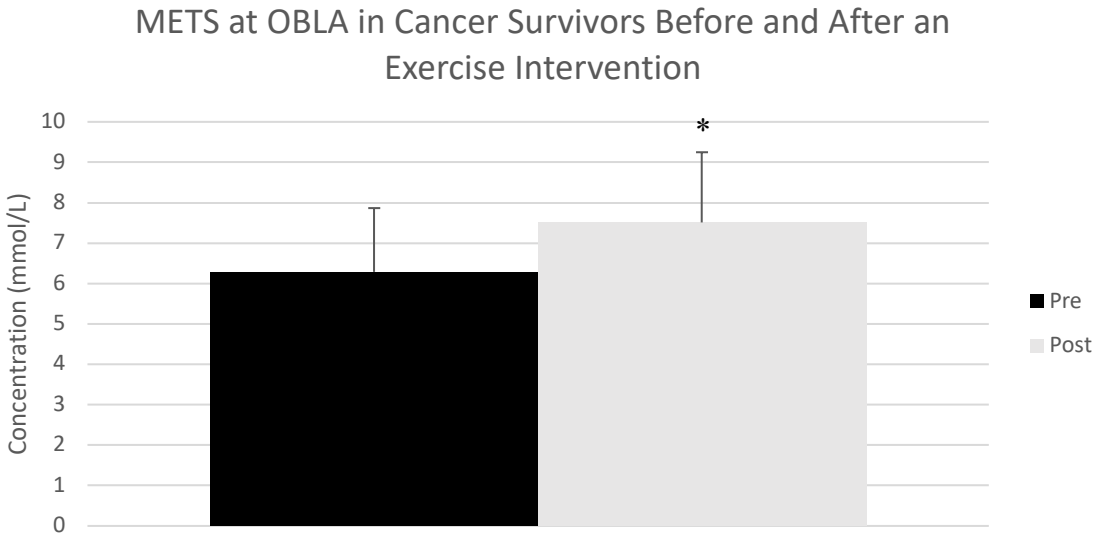

Figure 5: METS at OBLA in Cancer Survivors
METS (mL/kg/min) at OBLA for total subject population before and after a 12-week exercise-based oncology rehabilitation intervention. A two-tailed T-Test was used to test for evidence of significant differences. *P<0.05 vs. POST.

Stage Completion

Mean stage completion of active cancer survivors increased from 7.57 ± 2.92 to 9.57 ± 2.66 stages (p = 0.24) after a 12-week exercise-based oncology rehabilitation intervention (Figure 8). Mean stage completion of inactive cancer survivors increased from 8.86 ± 2.26 to 9.71 ± 2.76 stages (p = 0.39) after a 12-week exercise-based oncology rehabilitation intervention (Figure 9).

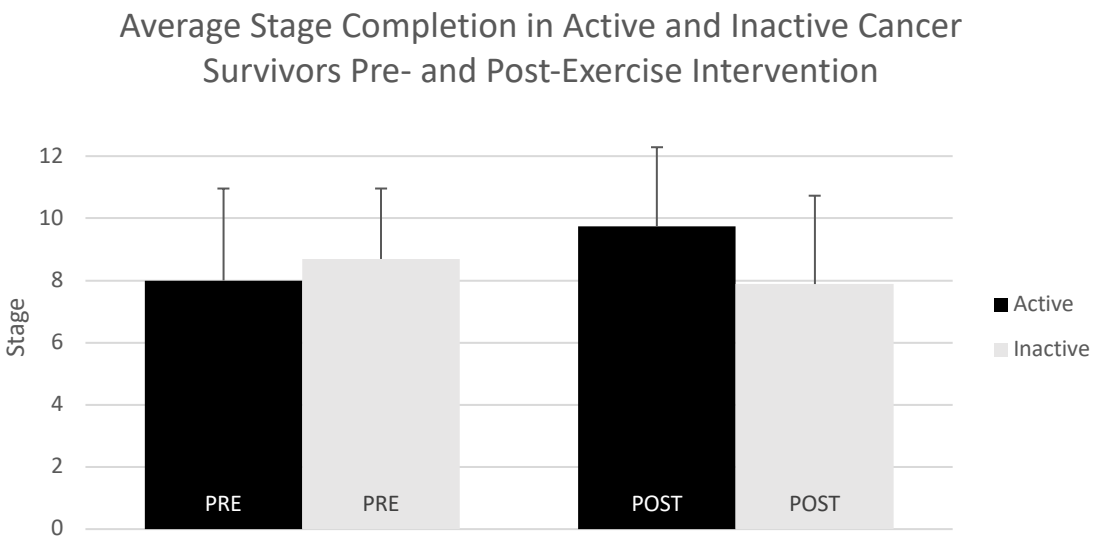

Figure 6: Stage Completion in Cancer Survivors
Pre (Left) and Post (Right) display mean stage completion with standard deviations in active (n=7) and inactive (n=14) cancer survivors before and after a 12-week exercise-based oncology rehabilitation intervention. No significant differences were observed.

Discussion

Studies on blood lactate parameters during exercise in a cancer population are lacking. As our understanding of lactate as an important precursor for tumorigenesis and a biomarker for evidence of metastasis and increased aggressiveness of cancer, it becomes all the more important to elucidate the mechanisms of how lactate handling can be affected in a cancer population. It is largely understood that exercise improves lactate handling in apparently healthy models, however the effects of exercise on lactate handling in a cancer population remains unclear.

Numerous studies indicate dysfunction and dysregulation in lactate handling in cancer survivors but how this can be attenuated through exercise is still unknown. Exercise interventions in cancer populations have shown similar significant

improvements in cardiovascular fitness and muscular strength, along with decreased fatigue and muscle wasting when compared to other diseased populations. No comparisons of blood lactate accumulation in cancer survivors and other populations exist, however. The present study demonstrates no significant differences in blood lactate accumulation between active and inactive before and after a 12-week exercise-based oncology rehabilitation intervention. A 12-week exercise-intervention for cancer survivors was able to improve buffering of lactate accumulation and subsequent exercise tolerance with increasing intensity of exercise, implying a regulatory approach to lactate production in cancer survivors. All participants in this study were able to safely complete this exercise protocol without any adverse effects.

Blood Lactate Handling

In the present study, changes in blood lactate accumulation (BLA) were quantified by comparing resting, peak, and METs at OBLA values between active and inactive cancer survivors. A 12-week oncology-based exercise rehabilitation intervention resulted in a lack of significant differences PRE/POST in resting ($P = 0.19, 0.26$), peak ($P = 0.97, 0.84$), and MET at OBLA ($P = 0.48$, $P = 0.77$) blood lactate accumulation levels between active and inactive cancer survivors respectively. However, significance in METS at OBLA was found before and after an exercise intervention between the entire population sample ($P = 0.04$), implying that the lack of significance between active and inactive populations could be due to an insufficient sample size. It is also notable that the lack of significance between active and inactive survivors could imply that groups may react to an exercise intervention in a similar fashion. This would indicate that typical cancer treatment may not play a role in lactate production in cancer survivors and further,

may not affect the way cancer survivors regulate lactate production, accumulation, and disposal upon the intervention of an exercise-based oncology rehabilitation program. A study conducted by Brizel et al., (2001) found that blood lactate concentrations of cancer survivors were up to 40% higher than that of an apparently healthy population. For apparently healthy individuals, blood lactate is normally 1-2 mmol/L at rest. In the present study, only the Active Survivors before (PRE) participating in the exercise intervention had a mean resting lactate value in excess of the normal range. This supports the notion that cancer may increase resting lactate since these individuals were undergoing treatments for active cancers. Whether high intracellular lactate levels of cancer cells translates to increased blood lactate was not determined in this study. Future studies should uncover the relationship between tumor lactate concentrations and blood lactate concentrations. Future studies may also benefit from deciphering the effects of an exercise intervention on resting lactate levels with a larger population size, as higher resting lactate levels are associated with cancer metastasis and poor prognosis.

METS are widely used as a clinical tool to estimate the energy expenditure of many different physical activities, where one MET is equal to 3.5 milliliters of oxygen consumed per kilogram of bodyweight per minute. A question posed here was related to whether an exercise intervention would allow cancer survivors to reach OBLA at higher a MET value after a 12-week exercise intervention. According to the study at hand, the cancer survivors that participated in this study did reach OBLA at significantly higher METS after a 12-week exercise intervention suggesting their ability to perform and effectively utilize oxygen to recycle lactate at higher intensities of exercise. OBLA levels, when compared pre- to post-exercise intervention, were lower at similar stages and

METS, treadmill protocol indicating improved exercise tolerance and lactate accumulation buffering as well as similar responses to exercise when compared to apparently healthy populations. Similar responses in active and inactive cancer survivors lacked significance, which may be due to an insufficient population size.

With a larger sample size, it is hypothesized that exercise would decrease resting lactate accumulation concentrations, increase peak lactate accumulation concentrations, and increase METS at OBLA, as it is seen in apparently healthy populations. These effects would indicate improved lactate handling in cancer survivors.

Potential Mechanisms of Lactate Handling in Cancer Survivors

The introduction of oxidative stress and chronic aerobic exercise training has been shown to lead to the upregulation of oxidative enzymes necessary for glycolytic catabolism and the subsequent utilization of glycolytic metabolites for oxidative phosphorylation. Endurance exercise-induced metabolic adaptations and/or resistance training-induced upregulation of MCT proteins facilitates improved lactate handling. Finding the difference between endurance and resistance exercise modalities on blood lactate accumulation in cancer survivors or pairing these exercise modalities together may maximize the potential to regulate lactate handling in cancer survivors thereby plausibly limiting the degree of acidosis induced angiogenesis and subsequent tumor metastasis and aggressiveness.

Recent studies indicate differences in cancer cell protein expression and metabolic behavior depending on the distance of cancer cells from the tumor vasculature. Cancer cells located farther from the tumor vasculature are inherently hypoxic and therefore highly glycolytic, expressing high levels of LDHA and MCT4 proteins thus facilitating

lactate production and extrusion into the tumor microenvironment. Cancer cells closer to the tumor vasculature are inherently oxidative, expressing high levels of LDHB and MCT1 proteins, collectively leading to a high affinity for lactate uptake and conversion to pyruvate for oxidative phosphorylation. This metabolic symbiosis is referred to as the "reverse Warburg effect," and postulates how metabolically self-sufficient tumors can be (Doherty & Cleveland, 2013; Whitaker-Menezes et al., 2011). It is speculated that catabolites and oxidative stress extruded into vasculature through exercise may starve tumors of nutrient and hypoxic factors (HIF-1α), resulting in decreased glycolytic intermediates and decreased lactate production as well as subsequent acidosis mediated angiogenesis.

In addition to exercise-mediated responses in improved lactate handling, exercise has also been noted to assist in proto-oncogenic and tumor-suppressor gene regulation. With far more literature justifying an effect of endurance training on carcinogenic gene regulation, it is plausible to imply that there are effects of exercise training on the regulation of glycolytic enzymes under- or overregulated by carcinogenic gene dysfunction either by exercise mediated gene regulation/repair or glycolytic protein (Sharafi & Rahimi, 2012). Furthermore, exercise mediated gene and glycolytic protein regulation would evidently shunt cancer mediated lactagenesis and improve lactate handling in cancer survivors (van Ginkel et al., 2016).

Limitations

Multiple studies describe a dysregulation in tumor intracellular lactate concentrations, however few address its relationship with blood lactate accumulation. As MCT proteins are upregulated in cancer populations, it is assumed that some portion of

lactate produced by tumors is extruded into the bloodstream. A limitation of this study is that tumor lactate concentrations were not quantified. Another limitation of this study includes the use of the Lactate Plus System, which is not FDA cleared for performing lactate measurements on persons for a medical intervention. The Lactate Plus System is intended for sports training and conditioning only. Furthermore, lactate concentrations were measured every two minutes during the treadmill protocol assessment, but due to the extensive monitoring of vitals during exercise testing, lactate concentrations were not measured every two minutes exactly. When pertaining to those severely compromised by their cancer and cancer treatment, some participants did not reach OBLA during the protocol, or OBLA was not obviously defined throughout their assessment. As such, if OBLA was not obviously measured, OBLA was assumed to be the concentration measured at the stage of or the stage before termination (OBLA met a ≥4.0mmol/L concentration criteria). In addition, the 12-week exercise intervention included both endurance and resistance training. Future studies should determine a difference in lactate handling, if any, between resistance training and endurance training in a cancer model.

Finally, the present study included participants of multiple cancer diagnoses and stages. As a result, the outcomes of this study may not apply to all cancer diagnoses and stages, since cancers can differ in both genotype and phenotype. Future studies should be conducted on homogeneous populations of cancer diagnoses and/or stages to collect accurate information on the production, accumulation, and disposal of lactate at rest and during exercise in cancer models.

CHAPTER IV

SUMMARY/CONCLUSION

Tumor cells are characterized as cells that undergo high rates of glycolysis excessive lactate formation, even in the presence of adequate oxygen (Warburg et al., 1927). It is well known that due to the collaborative dysfunction of proto-oncogenes and tumor-suppressor genes there is an upregulation of glycolytic enzymes and transport proteins that facilitate the catabolism of glucose and its subsequent excessive lactate production. Cancer survivors display dysregulation in how lactate is produced, accumulated, and disposed of and these notable dysregulations have been associated with increased cancer aggressiveness (Kunkel et al., 2003).

Blood lactate concentrations in cancer survivors have been found to be up to 40% higher than that of an apparently healthy population and lactate itself has been referred to as a "lactormone;" a lactate-mediated cell signaling molecule, due to its involvement in the upregulation of transport proteins and growth factors (Brizel et al., 2001; Brooks, 2009). Furthermore, current research in physiology emphasizes the effects of exercise on improved lactate handling *in vivo* largely due to the increased transcription of glycolytic, mitochondrial, and transport proteins.

However, research literature on blood lactate parameters during exercise in a cancer population is lacking. As lactate becomes an important precursor for tumorigenesis and a biomarker for evidence of metastasis and increased aggressiveness

of the cancer, it becomes all the more important to understand the mechanisms of how dysfunction of lactate handling can be attenuated in a cancer population.

Exercise interventions in cancer populations have found similar significant improvements in cardiovascular fitness, muscular strength and decreased muscle wasting, and other physiological parameters when compared to that of apparently healthy and other diseased populations. No comparisons of blood lactate accumulation in cancer survivors and other populations exist, however. The present study aimed to demonstrate differences in blood lactate accumulation between active and inactive cancer survivors before and after a 12-week exercise-based oncology rehabilitation intervention. A 12-week exercise-intervention for cancer survivors was able to improve exercise tolerance and subsequent buffering of lactate accumulation with increasing intensity of exercise, implying a regulatory approach to lactate production in cancer survivors. All participants in this study were able to safely complete this exercise protocol without any adverse effects.

Blood Lactate Handling

In the present study, changes in blood lactate accumulation (BLA) were quantified by comparing resting, peak, and Metabolic Equivalents at the Onset of Blood Lactate Accumulation (MET at OBLA) values between active and inactive cancer survivors. A 12-week oncology-based exercise rehabilitation did not significantly change blood lactate at rest ($P = 0.19, 0.26$), peak exercise ($P = 0.97, 0.84$), and MET at OBLA ($P = 0.48$, $P = 0.77$) between active and inactive cancer survivors respectively. Furthermore, significance in METS at OBLA was found before and after an exercise intervention between the entire population sample ($P = 0.04$), implying that the lack of

significance between active and inactive populations could be due to an insufficient sample size. It is also notable that the lack of significance between active and inactive survivors could imply that groups may react to an exercise intervention in a similar fashion. This would indicate that typical cancer treatment may not play a role in lactate production in cancer survivors and further, may not affect the way cancer survivors regulate lactate production, accumulation, and disposal upon the intervention of an exercise-based oncology rehabilitation program. A study conducted by Brizel et al., (2001) found that blood lactate concentrations of cancer survivors were up to 40% higher than that of an apparently healthy population. Future studies may benefit from deciphering the effects of an exercise intervention on resting lactate levels with a larger population size, as higher resting lactate levels are associated with cancer metastasis and poor prognosis.

METS equal the product of the amount of oxygen consumed/utilized multiplied by the resting value (3.5 ml/kg/min) and so a question to help elaborate on improved lactate handling during an exercise intervention in cancer survivors is, “did cancer survivors reach OBLA at higher METS after a 12-week exercise intervention?” According to the study at hand, the 21 cancer survivors that participated in this study did significantly reach OBLA at higher METS after a 12-week exercise intervention implying their bodies are capable of performing and effectively utilizing oxygen to recycle lactate at higher intensities of exercise. OBLA levels, when comparing pre- to post-exercise intervention, were lower at similar stages and METS, of the UNCCRI treadmill protocol indicating improved exercise tolerance and lactate accumulation buffering as well as similar responses to exercise when compared to apparently healthy

populations. Similar responses in active and inactive cancer survivors lacked significance, which may be due to an insufficient population size.

With a larger sample size, it is hypothesized that exercise would decrease resting lactate accumulation concentrations, increase peak lactate accumulation concentrations, and increase METS at OBLA, as it is seen in apparently healthy populations. These effects would indicate improved lactate handling in cancer survivors.

Potential Mechanisms of Lactate Handling in Cancer Survivors

Current literature has shown exercise mediated regulations of proto-oncogenes and tumor-suppressor genes particular to lactate production in cancer survivors. With far more literature proposing endurance training versus resistance training-mediated regulation of these genes, included are HIF-1α, c-Myc, and p53, which when susceptible to dysfunction collaborate to promote the upregulation of glycolytic mechanisms. As a result, tumor microenvironments are characterized as high in glucose influx and high in lactate production, accumulation, and disposal. Skeletal muscle contraction induces increased rates of flow throughout the vasculature. Upon the initiation of exercise, increased rates of skeletal muscle contraction follows, as does skeletal muscle contraction mediated blood flow in the attempt to adequately supply nutrient and eliminate waste to and from metabolizing tissue as is seen in apparently healthy populations (Jorfeldt & Wahren, 1971; Lash, 1996). Theoretically, through exercise mediated increases in blood flow, it is hypothesized that exercise may redirect the nutrient typically being perfused to a tumor towards working tissues during exercise. Additionally, it is plausible to assume that lactate, as a cell-signaling molecule, would be removed from the tumor

microenvironment as well, preventing acidosis induced angiogenesis responses to lactate production.

Upon higher intensities of exercise, the body is introduced to oxidative stress, or the inability of tissues to eradicate reactive oxygen species efficiently. The introduction of oxidative stress and chronic aerobic exercise training has been shown to lead to the upregulation of oxidative enzymes necessary for glycolytic catabolism and the subsequent utilization of glycolytic metabolites for oxidative phosphorylation. With an increase in flow, there is an increase in oxidative stress introduced to a tumor microenvironment, which is heavily glycolytic and unable to attenuate to the reactive oxygen species accumulating throughout the vasculature. Miyata, Matsuo, Sagara, Ohba, Ohyama, & Sakai (2017), have found that introducing reactive oxygen species to the tumor microenvironment can induce apoptosis, or programmed cell death.

In addition to flow-mediated applications of lactate handling in cancer populations, there is evidence of improved lactate handling with both endurance and resistance-based exercise modalities. Through the upregulation of MCT and glucose transport proteins, as well as glycolytic intermediates necessary for oxidative phosphorylation, individuals can tolerate exercise at higher intensities through the utilization of oxidative energy-yielding mechanisms and subsequent lactate buffering. This study hypothesized that after a 12-week exercise intervention, a cancer survivor would be able to handle lactate production, accumulation, and disposal more efficiently. However, it is unclear whether these exercise-mediated effects in lactate handling were due to endurance training, resistance training, or a combination of both. Finding the difference between endurance and resistance exercise modalities on blood lactate

accumulation in cancer survivors or pairing these exercise modalities together may maximize the potential to regulate lactate handling in cancer survivors thereby plausibly limiting the degree of acidosis induced angiogenesis and subsequent tumor metastasis and aggressiveness.

Recent studies indicate differences in cancer cell protein expression and metabolic behavior depending on the distance of cancer cells from the tumor vasculature. Cancer cells located farther from the tumor vasculature are inherently hypoxic and therefore highly glycolytic, expressing high levels of LDHA and MCT4 proteins thus facilitating lactate production and extrusion into the tumor microenvironment. Cancer cells closer to the tumor vasculature are inherently oxidative, expressing high levels of LDHB and MCT1 proteins, collectively leading to a high affinity for lactate uptake and conversion to pyruvate for oxidative phosphorylation. This metabolic symbiosis is referred to as the "reverse Warburg effect," and postulates how metabolically self-sufficient tumors can be (Doherty & Cleveland, 2013) (Whitaker-Menezes et al., 2011). It is speculated that catabolites and oxidative stress extruded into vasculature through exercise-mediated increases in flow may starve tumors of nutrient and hypoxic factors (HIF-1α), resulting in decreased glycolytic intermediates and decreased lactate production as well as subsequent reactive oxygen species mediated apoptosis.

Limitations

Multiple studies describe a dysregulation in tumor intracellular lactate concentrations, however few address its relationship with blood lactate accumulation. As MCT proteins are upregulated in cancer populations, it is assumed that some portion of lactate produced by tumors is extruded into the bloodstream. A limitation of this study is

that tumor lactate concentrations were not quantified. Another limitation of this study includes the use of the Lactate Plus System, which is not FDA cleared for performing lactate measurements on persons for a medical intervention. The Lactate Plus System is intended for sports training and conditioning only. Furthermore, lactate concentrations were measured every two minutes during the treadmill protocol assessment, but due to the extensive monitoring of vitals during exercise testing, lactate concentrations were not measured every two minutes exactly. When pertaining to those severely compromised by their cancer and cancer treatment, some participants did not reach OBLA during the protocol, or OBLA was not obviously defined throughout their assessment. As such, if OBLA was not obviously measured, OBLA was assumed to be the concentration measured at the stage of or the stage before termination (OBLA met a ≥4.0mmol/L concentration criteria). In addition, the 12-week exercise intervention included both endurance and resistance training and it is unclear whether exercise-mediated effects on lactate response in cancer survivors during an exercise bout to volitional fatigue are due to endurance training, resistance training, or a combination of both.

Finally, the present study included participants with a range of cancer diagnoses and stages. As a result, the outcomes of this study may not apply to all cancer diagnoses and stages, since cancers can differ in both genotype and phenotype. Future studies should be conducted on homogeneous populations of cancer diagnoses and/or stages to collect accurate information on the production, accumulation, and disposal of lactate at rest and during exercise in cancer models.

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APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL

UNIVERSITY OF
NORTHERN COLORADO

Institutional Review Board

DATE:	March 28, 2019
TO:	Reid Hayward, PhD
FROM:	University of Northern Colorado (UNCO) IRB
PROJECT TITLE:	[573297-7] Exercise Interventions to Attenuate the Negative Side-Effects of Cancer Treatments
SUBMISSION TYPE:	Continuing Review/Progress Report
ACTION:	APPROVED
APPROVAL DATE:	March 28, 2019
EXPIRATION DATE:	March 28, 2020
REVIEW TYPE:	Expedited Review

Thank you for your submission of Continuing Review/Progress Report materials for this project. The University of Northern Colorado (UNCO) IRB has APPROVED your submission. All research must be conducted in accordance with this approved submission.

This submission has received Expedited Review based on applicable federal regulations.

Please remember that informed consent is a process beginning with a description of the project and insurance of participant understanding. Informed consent must continue throughout the project via a dialogue between the researcher and research participant. Federal regulations require that each participant receives a copy of the consent document.

Please note that any revision to previously approved materials must be approved by this committee prior to initiation. Please use the appropriate revision forms for this procedure.

All UNANTICIPATED PROBLEMS involving risks to subjects or others and SERIOUS and UNEXPECTED adverse events must be reported promptly to this office.

All NON-COMPLIANCE issues or COMPLAINTS regarding this project must be reported promptly to this office.

Based on the risks, this project requires continuing review by this committee on an annual basis. Please use the appropriate forms for this procedure. Your documentation for continuing review must be received with sufficient time for review and continued approval before the expiration date of March 28, 2020.

Please note that all research records must be retained for a minimum of three years after the completion of the project.

If you have any questions, please contact Nicole Morse at 970-351-1910 or nicole.morse@unco.edu. Please include your project title and reference number in all correspondence with this committee.

Continuation materials are approved. Best wishes with the continued work on this project.